A STUDY OF PROTEIN EXTRACT FROM SOYBEANS WITH REFERENCE TO ITS USE IN FOOD[1]

By SYBIL WOODRUFF, *chief in foods*, EVELYN CHAMBERS, *graduate student*, and HELEN KLAAS, *first assistant in home economics, Illinois Agricultural Experiment Station*

INTRODUCTION

The soybean (*Soja max* (L.) Piper) is unique among plant foods in that it contains about 40 percent of protein. The supply of soybeans gives promise of being abundant, too; Illinois produced in 1937 a little less than 22 million bushels, an increase of about 27 percent over her crop for 1936. Here is a potentially important source of protein, little of which is now being consumed as food. Some people are advocating that the United States extend the food uses of soybeans for the sake of obtaining inexpensive protein. This might be done in either of two ways; by utilizing the beans themselves or by making from the beans a protein concentrate which could take its place with other protein-rich foods.

The purpose of this study was to find a simple method of extracting semipure protein from soybeans and then to determine whether the protein substance could be used advantageously in the preparation of food.

REVIEW OF LITERATURE

The literature relating to soybean proteins and their physical behavior is not extensive and is somewhat confused by the variety of laboratory methods which have been used in extracting the proteins. Moreover, papers bearing on the topic have not been concerned with the uses of the protein in food technology but rather with its chemical nature. There is little in the literature concerning the methods that are being used for removing crude soybean protein for the manufacture of plastics. A recent paper by O'Brien (*10*)[2] gives methods and factory costs for such industrial operations.

Osborne included soybeans in his classic studies of vegetable proteins. In 1898 he and Campbell (*12*) suggested the name "glycinin" for the globulin they dissolved from soybeans with a 10-percent sodium chloride solution. This solvent has continued to be employed more often than any other in extracting globulins. The conventional definition of a globulin would exclude protein extracted by any other solvent than dilute neutral solutions of salts of strong bases with strong acids. Yet O'Hara and Saunders (*11*) have recently reported success in extracting crystalline protein having the characteristics of a globulin with either saturated or normal solutions of sodium chloride. They used orange seed, peanut, and other proteins, but their conclusion that the "ordinary text book definitions of globulins do not adequately consider the solubility properties of globulins" is concurred in by other writers who have worked with still other materials (*4*).

It has been commonly observed that soybean proteins are readily soluble in water. Osborne and Campbell (*12*) said the fact that as

[1] Received for publication March 11, 1938; issued December 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 746.

much as 16 percent of the glycinin could be dissolved in water was due to the presence of potassium phosphates in the seed. Both Nakajima (*9*) and Muramatsu (*8*) showed that if soybeans were extracted first with water, there was little remaining protein which would dissolve further in sodium chloride or sodium hydroxide solutions. The last-named author has divided the water-soluble nitrogen of soybeans as follows: Globulins (largely glycinin), 84.25 percent; albumins, 5.36 percent; proteose and nonprotein nitrogen, the remainder.

The usual methods mentioned in the literature for recovering the protein in solid form are dialysis to remove salt and treatment of the extraction liquor with either ammonium sulphate or acid. Hartman and Cheng (*2*, *6*) gave the isoelectric point of soybean protein as pH 5.00. In almost every case reported in the literature the protein product has been dried with alcohol and ether; Hartman and Cheng (*5*) recommended methyl alcohol and ether.

Comments on solubility usually pertain to the ease of dissolving the protein from the seed, not to the solubility of the separated, dried protein. Denatured glycinin was said by Tadokoro and Yoshimura (*13*) to be most soluble in 0.1 to 0.25 normal sodium hydroxide though it was also soluble in several acids. They also found that heating soybeans caused a large percentage of their proteins to be less soluble in water and more soluble in alkaline solutions. Gortner (*3*) believed solubility of globulins to be merely peptization and governed by the ions present. Nevertheless the solubility of glycinin has been variously reported and likewise its ability to coagulate.

METHODS OF EXTRACTION

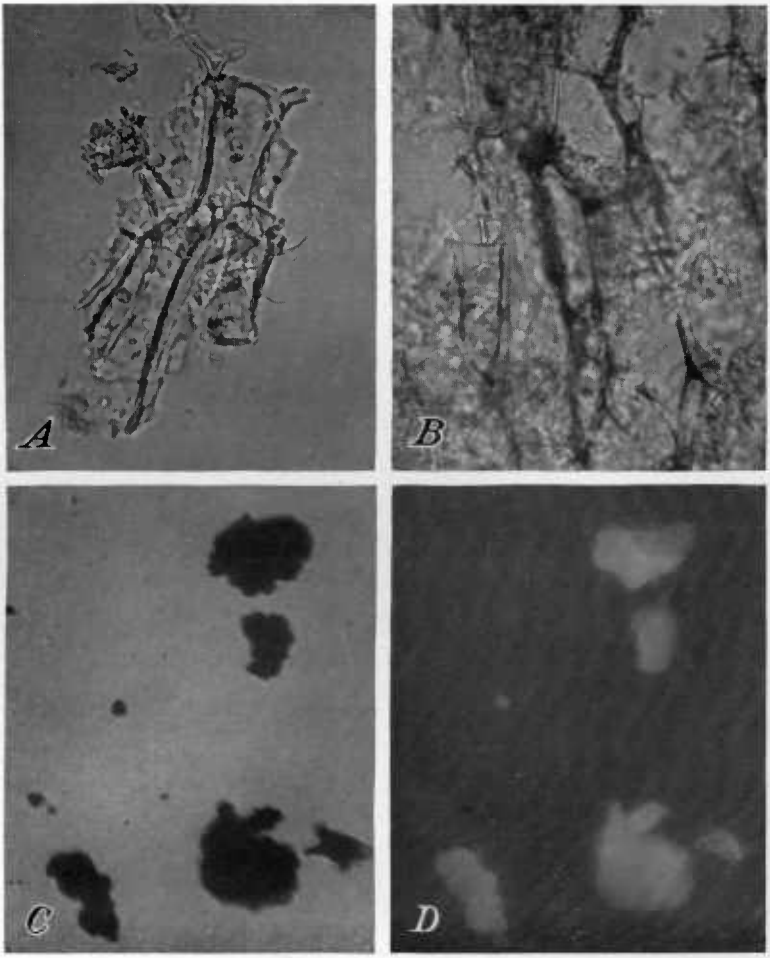
Mature soybeans of two "vegetable" types were used in the experiments, identified throughout the paper by serial Nos. 85666 and 81029. Both had been found in other work (*15*) with soybeans to be satisfactory for food uses. Without preliminary drying, each was ground in a Bauer mill to pass a 60-mesh screen; then extracted continuously for 24 hours with petroleum ether (boiling point 30° to 60° C.) and further ground in a porcelain mortar to pass a 100-mesh sieve. Previous heating of the soybeans was avoided because it tended to make the proteins less soluble.

Commercial fat-free soybean flakes [3] were used for much of the routine experimenting with solvents and methods. They were made from No. 2 Illini soybeans and had been treated only with organic solvent.

SOLVENT

Water was found to be the most practical solvent for protein extraction from the standpoint of yield and purity of product, and time and equipment involved. To 100 g of ground fat-free soybeans was added 750 ml of distilled water, and the suspension was agitated gently in a mechanical shaking machine for 30 minutes at room temperature. It was next poured into a canvas bag and the water "solution" pressed from it in a tincture press. The soybean residue was twice more extracted with fresh portions of 750 ml of water. Suspended matter was separated from the extracts by centrifuging, and the resulting "solutions" became less opaque and viscous in succeeding extractions. Little success was attained in filtering the

[3] Obtained from the Glidden Co., Chicago.



Microscopic appearance of soybean materials: *A*, Fat-free soybean flakes, ground to pass a 60-mesh screen; *B*, residue of flakes after extractions were completed, showing some cells still intact; *C*, soybean protein substance mounted dry showing dense masses of material; *D*, same field as *C* examined between crossed Nicols. Note the luminous character of the protein masses, probably anisotropic. × 900.

extract even with a special filter of acid-alkali-treated asbestos and paper pulp which had been recommended by Hartman and Cheng (*5*). The liquors of the different extractions were kept separate and each in turn was diluted to 800 ml for precipitation of protein.

The commercial flakes absorbed less water than the soybeans, hence only 500 ml of water was used for their first extraction and 400 ml for the second and third. The extracts were diluted to 500 ml before the protein was precipitated.

Solvents other than water were used in a series of preliminary trials with commercial soybean flakes. Solutions of sodium chloride, sodium carbonate, and sodium hydroxide were used in turn. Sodium chloride in 10-percent solution had been frequently used by other investigators; it might well be expected to be the ideal solvent for the principal proteins of soybean, thought to be globulin in nature. Salt solution was not found to be superior to water as a solvent for the purposes of this experiment.

PRECIPITATING AGENT

Dilute acetic acid (about 1 or 2 ml) was added to each water extract of protein, and when the acidity reached pH 5.0 the protein precipitated as a white cloud. Hydrochloric was found to be no more successful than acetic acid. The addition of sufficient calcium chloride to make the liquor 0.02 molar with respect to the salt, aided in precipitating the protein, but this method likewise had no advantage over that of precipitating it with acetic acid. Dialysis through collodion bags effected a satisfactory precipitation of protein from the liquor extracted with sodium chloride solution.

The precipitate settled readily, after which the waste liquor was decanted and centrifuged from it. Successive portions of 70-percent, 95-percent, and then absolute ethyl alcohol were used to wash and dry the protein. It was last washed with ether and worked to dryness on a porous plate. The product was a white, fine powder which showed no tendency to gumminess after it had thus been thoroughly dried. Drying only in air gave a horny substance. No special advantage was found in drying the material with methyl alcohol as recommended by Hartman and Cheng (*5*).

YIELDS

About 52.6 percent of all the nitrogen originally present in the fat-free soybeans was recovered when the protein substance was dissolved in water and precipitated by acetic acid in three successive treatments. A fourth extraction was found not to add significantly to the total yield. The amount of protein left behind in the residue was about 22 percent of the quantity originally present. The fraction that failed to precipitate when acetic acid was added was about 15 percent of the original soybean protein. Unaccountable loss amounted to about 10 percent of the total protein. This waste, thought to be due almost wholly to seepage of the finely ground soybeans through the canvas bag, could be reduced by grinding the beans to pass a 60-mesh screen instead of a 100-mesh. The saving thus effected compensated for the slightly lesser solubility of the protein of the more coarsely ground beans.

Photomicrographs of the original sample, ground to pass a 60-mesh screen, and of the same after it had been extracted three times with water show (pl. 1, *A* and *B*) that it was probably the presence of intact cells which interfered with complete solution of the protein contained

in them and not any inherent insoluble nature of a part of the protein. The photograph also illustrates well the location of the protein and other material in relation to the cells.

ANALYSES

The composition and therefore the purity of the protein substance was found on analysis to vary with different methods of extraction. Nitrogen was determined by the Kjeldahl method; the ashing temperature was 600° C.; samples were dried for moisture determinations at 80° C. in vacuo for 24 hours.

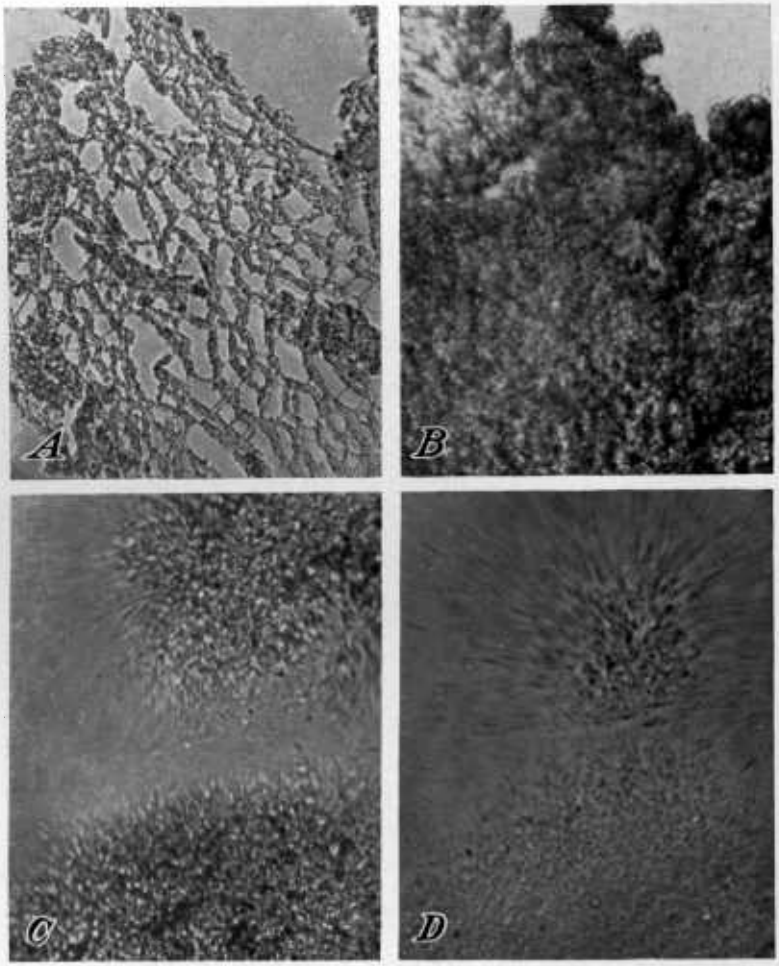
The protein substance yielded by dissolving with water and precipitating with acid, had the highest nitrogen content (16.13 percent) of all samples with the exception of the one dissolved in sodium chloride and dialyzed. The yield by the latter method was small, however. Other authors have reported the nitrogen content of protein substance, obtained by means of various solvents, to be from 12.25 to 17.53 percent. Data in table 1 show that samples obtained with other solvents were less exclusively protein in content; it was largely for this reason that water was selected as the most practicable solvent. The highest yield of protein substance ever obtained was with sodium hydroxide in 0.2-percent solution; but analysis of the material showed it to contain a high percentage of nonprotein constituents, particularly ash.

It is possible that hemicellulose material was dissolved by the alkali treatment just mentioned and was in turn precipitated by the acid used in separating the protein from solution. Occurrence of this kind may even have been observed to some degree when water was used as a solvent. The results given in table 2 show that 39 g of material out of the original 93.6 g of moisture-free beans could be accounted for in neither the protein substance nor the residue of soybeans left after three extractions were made. Of this 39 g of material unaccounted for, 22.78 g were shown by the data to be neither protein nor ash. It is assumed that this unidentified material dissolving out from the soybeans was largely carbohydrate in nature. It seems remarkable that the quantity of water-soluble constituents is as high as this. Further identification of water-soluble carbohydrates is under way.

TABLE 1.—*Analyses of protein substance extracted from soybeans by various methods*

Extraction method		Kind of soybeans	Protein substance		Constituents in moisture-free protein substance			
Solvent	Means of precipitating		Yield [1]	Moisture content	Ash	Nitrogen	Protein	
							N×5.71	N×6.25
			Grams	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Water	Acetic acid	No. 85666 [2]	24.75	5.44	2.34	16.23	92.7	101.4
		No. 81029 [3]	27.37	6.00	2.05	16.13	92.1	100.8
		Commercial flakes.[4]	25.27	6.83	2.24	16.03	91.1	100.2
Sodium chloride solution, 10 percent.	Dialysis	do	10.35	5.78	.57	16.35	93.3	102.2
	Acetic acid	do	13.21	5.63	1.22	13.24	75.6	82.7
Sodium carbonate solution, 10 percent.	do	do	10.36	9.85	4.92	12.55	71.7	78.4
Sodium hydroxide solution, 0.2 percent.	do	do	35.58	4.98	1.21	13.57	77.5	84.8
	Hydrochloric acid.	do	19.83	7.36	1.79	13.00	74.2	81.2
Sodium hydroxide solution, 10 percent.	do	do	10.63	5.92	20.22	13.80	78.8	86.2

[1] From 100 g of fat-free soybeans.
[2] Average of 3 runs.
[3] Average of 2 runs.
[4] Average of 7 runs.



Microscopic appearance of protein substance during precipitation and during peptization: *A*, Threads or chains of newly precipitated protein substance in the mother liquor; *B*, dried protein substance mounted in water, photographed after 10 minutes to show that no peptization had occurred; *C*, dried protein substance mounted in 0.025-percent NaOH solution, after 1 minute standing, shown after peptization had started; *D*, same field as *C* after 10 minutes standing when most of the material had peptized. × 900.

TABLE 2.—*Nonprotein constituents removed from soybeans by extraction with water*

[Amounts based on 100 g of fat-free soybeans No. 85666]

Material	Air-dried weight	Moisture-free weight	Constituents present		
			Ash	Proteins N×5.71	Others by difference
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
Fat-free soybeans	100	93.6	6.32	45.11	42.16
Protein substance	24.7	23.4	.55	21.69	1.16
Residue	34.0	31.2	.79	12.20	18.22
Loss in waste liquor, etc.	----------	39.0	4.98	[1] 11.22	22.78

[1] Analysis of the waste liquor accounted for 6.05 g of this protein loss.

PROTEIN CONVERSION FACTOR

In recording the protein equivalent of the nitrogen percentages found by analysis, the factor 5.71 has been employed, since Jones (*7*) found it to be the correct one for soybeans. Few investigators even mention the conversion factor they have used, though it appears likely from their results that usually they have used 6.25. That the factor 6.25 was incorrect for the nitrogen yields of this study is indicated in table 1, where the protein content of the substance is shown as computed with each factor. The factor 6.25 made the protein appear to be more than 100 percent of the weight of substance. The protein content of the moisture-free protein substance was 92 percent (N×5.71).

SOYBEAN PROTEIN SUBSTANCE

The general physical behavior of the protein substance was a matter of major concern in the study because the usefulness in food technology of such a concentrated form of protein would be determined by properties like solubility, ease of coagulation, and behavior as a colloid. Several tests designed to reveal its potential functioning in this food capacity were carried out. Samples extracted from the three varieties of soybeans showed no recognizable differences in general behavior.

Separation and identification of the proteins which were precipitated from the water extractions was not one of the purposes of this study. The term "protein substance" has been used by the authors to refer to the recovered material because it was thought unlikely that only an individual protein was present. If other writers are correct, the protein substance was largely the globulin glycinin. It contained, as the above-mentioned analyses indicated, some moisture, a small amount of ash, and about 5 percent of unidentified material.

APPEARANCE AND SOLUBILITY

The protein substance was a tasteless, odorless white powder, the presence of which would be wholly unobjectionable as an addition to foods. So far as could be told, it was not crystalline. During the process of its precipitation from the liquor, microscopic threadlike chains or networks were characteristically present. Such an appearance is shown in plate 2, *A*. During drying the clumps assumed an appearance which would have been described as amorphous except

for the fact that under the microscope they were luminous when examined dry between crossed Nicols. It seemed possible that each clump was made up of innumerable anisotropic units; their luminosity could not be extinguished as the Nicols were turned, but this might have been due to the countless anisotropic units assuming as many different positions. Plate 1, *C* and *D*, shows masses of protein substance mounted dry and examined without and with crossed Nicols.

In water, the clumps exhibited no change in microscopic appearance which could be attributed to dissolving action, though anisotropy did not exist. This is shown in plate 2, *B*, where the clumps remained unchanged after standing in water for 10 minutes. The substance was seen to disintegrate rapidly when it was suspended in a very dilute sodium hydroxide solution (0.025 percent) and watched for several minutes under the microscope. Plate 2, *C* and *D*, shows that the protein clumps were peptizing even after standing 1 minute in the dilute alkali and had almost disappeared after 10 minutes.[4]

But little of the protein substance could be redissolved in water or even in sodium chloride solution. This was true while the material was still moist and also after it had been dried with alcohol. The protein substance was suspended in the solvent and shaken at room temperature for 1.5 hours, at the end of which time only about 12 to 20 percent of it had disappeared. It gave no appearance of dissolving in water though it was peptized readily by either 10-percent sodium carbonate or 0.025-percent sodium hydroxide solutions. The globulin had obviously undergone a transformation to an insoluble protein during the precipitation process. The protein may well have been further denatured during drying, but whether it was greatly exaggerated by alcohol treatment, was not determined.

Both 10-percent sodium carbonate and 6 N acetic acid solutions peptized or softened the protein to a gelatinous mass in a short time. Either of the last two solvents might safely be used in less concentrated solutions for such purpose in food preparation. The dried protein substance, originally extracted from the soybeans with alkaline solutions, dissolved to a somewhat greater extent than the one obtained by the method adopted. But this fact was probably explained by the presence of significant amounts of nonprotein impurities in the protein substance obtained by such solvents.

FOAMING ABILITY

The marked tendency of soybean suspensions to foam was a troublesome property in the processes of extraction, but on the other hand, it was seen to have certain possibilities of practical application. Several tests were made to determine whether the protein substance itself had any foaming ability with the idea in view that it might be put to use in food technology wherever egg white or gelatin are now employed for such purposes.

Eight grams of material, either ground soybeans, or protein substance, or residue from the extractions, in 100 ml of water was whipped at high speed in an electric mixer for 8 minutes. The volume of the foam if one formed at all was measured and then it was emptied into a large funnel containing two layers of cheesecloth. The volume of fluid which had drained from the foam at stated intervals was meas-

[4] Acknowledgment is made of the assistance of Dr. Majel M. MacMasters, associate in home economics, in the preparation of the photomicrographs.

ured as an indication of foam stability, more liquid draining from the less stable foam. The foaming ability of the liquors was measured by treating 100 ml of the liquor as above. The results of the foaming test are given in table 3.

TABLE 3.—*Volume and stability of soybean liquor foams*

Material	pH of solution	Volume of foam [1]	Water drained from foam [1] in—		Remarks
			5 minutes	30 minutes	
		Milliliter	*Milliliter*	*Milliliter*	
Fat-free soybean [2]		600	4	28	Heavy foam, thick consistency.
Residue, moist [2]	6.0	250	70		Thick paste, little foam.
Residue, dried [2]		(3)			
Protein substance:					
Used while still moist [2]		(3)			
Used after dried [2]		(3)			
Protein liquors, first extractions:					
Before precipitation of protein	6.1	1,200	0	60	Stable foam.
After precipitation of protein	5.0	1,600	2	60	Stiff, dry foam.
After precipitation of protein and addition of Na_2CO_3.	6.0	1,400	4	65	Foam like egg white.
After precipitation of protein, followed by removal of heat-coagulable proteins.	5.0	1,700	40	80	Do.
Protein liquors:					
First extraction, after precipitation	5.0	1,600	5	70	Stiff, dry foam.
Second extraction, after precipitation	5.0	1,100	40	85	Stiff foam.
Third extraction, after precipitation	5.2	500	75	85	Thin, watery foam.

[1] Foam produced by 100 ml of solution or suspension.
[2] A suspension contained the equivalent of 8 g of air-dry material per 100 ml.
[3] No foam.

Liquors remaining after most of the protein substance had been precipitated produced fairly stable foams of large volume. One such sample also became ropy and gelatinous after standing overnight in the refrigerator. The presence of mucilaginous constituents has commonly been observed in this laboratory in both green and mature soybeans. In fact, the liquor present in canned green beans, processed at 10 pounds pressure, has usually been found to appear as a soft jelly. No one pH value seemed to be required for the foaming, and the amount of protein dissolved in the liquor did not determine the volume of the foam. With a diminution in amounts of all constituents in succeeding extractions, the volume and stability both decreased.

The soybean residue from the extractions had practically no foaming power in spite of the fact that it still contained about 37 g of protein in 100 g. of moisture-free material. The original fat-free soybeans, on the other hand, formed a fairly stable foam of small volume; part of its stability seemed to be due to suspended matter, for its consistency was very different from that of the other foams.

The results showed that the protein substance, having undergone transformation to an insoluble form during its precipitation, had no ability to produce a foam either when used as freshly precipitated, still moist material or when suspended in water again after it had been dried. Watts (*14*), who investigated the whipping ability of ground, defatted soybeans, had attributed the foams obtained to protein contained by the flour. It is the opinion of the authors that other soybean constituents, e. g., saponins, pectinous or gumlike carbohydrate

derivatives, are responsible to a great degree for the foaming of the ground soybeans and of the liquors. Carbohydrates and like constituents are in process of investigation in the authors' laboratory now. The presence of saponins in soybeans has been reported by several groups of investigators, one of whom, Burrell and Walter (*1*), have recently prepared a crystalline saponin from soybean meal and studied its behavior.

OTHER PHYSICAL PROPERTIES

The protein substance differed from ground fat-free soybeans in several points of behavior. For one thing, it failed to absorb water when moistened and allowed to stand. One of the outstanding characteristics of soybean flour is its ability to absorb and hold large amounts of water. For example, a commercial low-fat soybean flour has been found to take up about 2.5 times its weight of water. This was determined by centrifuging off at high speed all surplus water which the moistened flour failed to retain. By the same test the residue of soybeans left after the proteins were extracted was found to have doubled the original capacity of soybeans to hold water. Milliliters of water absorbed by 100 g of material were as follows:

	Milliliters
Commercial soybean flour, low-fat	245
Commercial soybean flakes, fat-free	255
No. 85666 soybeans, fat-free	225
Residue from No. 85666 soybeans after protein extraction	490

These results indicated that other constituents, probably carbohydrate in nature, were responsible to a large degree for the swelling of soybean products in water.

Seed globulins have usually been reported in the literature to be not heat-coagulable. There is evidence of their slow rate of coagulating in the fact that "soybean milk" can be boiled for several minutes without visible change. Soybean milk is the name given a milky-white, watery suspension which is in common use in the Orient. Tests on the water extracts obtained in these experiments showed that the protein present did not coagulate by heat so long as the pH value was 6.0. After an addition of acetic acid had reduced the pH value to 5.00 and the precipitated protein had been removed, the remaining liquor allowed successive coagula to form at 66°, 80°, and 96° C. as heating and filtering were continued. But in all, the weight of heat-coagulated proteins was only about one-fifteenth of that of the protein known from the analyses to have been present in the liquor.

The very slight solubility of the protein substance has been mentioned already. The small amount which did dissolve, either as freshly precipitated or previously dried protein, showed no visible coagulation even when heated to boiling.

VALUE AS A PROTEIN SUBSTITUTE IN COOKERY

The protein substance being a tasteless white powder was found to have no objectionable features as an article of food. Several tests were made for the purpose of determining whether it had advantages in behavior as a colloid. A few typical dishes were prepared to learn whether it could be satisfactorily substituted for the usual weight of egg protein used for thickening purposes. In a custard no thickening whatever was observed whether the soybean protein was boiled with

the milk or allowed to cook at the lower temperature of the usual baked custard. This was true when both dried and freshly precipitated protein substance were used. Salts or other constituents of the milk, therefore, did not favor peptization of the soybean protein. Muffins were made to contain egg protein, or soybean protein substance in both dried and freshly precipitated forms, or no protein of either kind. The products made with soybean protein were neither better nor worse than those made with no egg at all. The ones containing egg were superior, however, to those without egg. Soybean flour has only slight thickening or binding quality, hence but little of this property has been lost in the conversion of flour to protein substance.

The soybean protein was found to have two advantages over soybean flour; it contained more than twice as much protein and it was free from flavor. Even though it was not found to be a successful substitute for animal proteins for cooking purposes, its presence in a mixture did not interfere with the expected reactions of other components. There is no reason to believe that its digestibility and utilization in the body have been lessened by converting it into a difficultly soluble form of protein during the process of extracting it from the soybean.

It is suggested that soybean protein might be incorporated with any food of moderately thick consistency if there is an advantage in thus increasing the protein content of the diet. Cereals, thickened soups, vegetable dishes, and escalloped foods of many kinds might have the protein substance incorporated in rather large amounts. Further work may reveal ways of reducing the amount of denaturation of the protein as it is precipitated, dried, and stored. Possibly it might then be used still more effectively in food preparation.

SUMMARY

The purpose of the study was to find a simple method of extracting semipure protein from soybeans and then to determine whether the protein substance could be used advantageously in food preparation.

Protein was extracted from two varieties of soybeans and from commercial soybean flakes by treating fat-free finely ground beans with water at room temperature and then precipitating the protein from the extract by acidification with acetic acid to pH 5.0.

The dried protein substance thus obtained represented about 52.6 percent of the nitrogen originally present in the soybeans. It was 92 percent protein ($N \times 5.71$) on a moisture-free basis.

The protein substance was not crystalline but appeared to be anisotropic. Photomicrographs show its luminous appearance between crossed Nicols and also show it in the process of precipitating and peptizing.

It was not measurably soluble in water or salt solution, though acetic acid and sodium carbonate solutions caused it to soften and swell. Suspensions of it did not foam; in this behavior it differed markedly from suspensions of ground fat-free soybeans, the foaming ability of which is probably due partly to nonprotein constituents.

The protein substance did not produce a thickening or binding of food ingredients similar to the effect caused by egg proteins in custards or muffins. Neither did its presence interfere with expected reactions of other components during cooking. It was tasteless and wholly unobjectionable as an addition to other foods.

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It has been suggested that the protein substance might be incorporated in many food dishes for the purpose of adding to their protein content. The substance is thought to offer possibilities as a new source of food protein, and to have the advantage over soybean flour of higher protein content and freedom from flavor.

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